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(2013 / 4 / 29 2013/ 2 / 17)

(LP)

G-100 G-50

1000± 76493

900± 75219

(G-100)

/ 70

25 7

/ 0.08

-)

/ 400 ° 45

3.49

(K_m)

(V_{max})

(

45.4 /

4.44

/ 26.3 /

/

(NaAsO₂)

(Phenylphrine)

(Na₂-EDTA)

:

Separation and Studies of Lactoperoxidase from Camel and Buffalo Milk Locality

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ABSTRACT

The research included the separation of lactoperoxidase (LP) from camel and buffalo milk locality using different biochemical techniques. One proteinous band had been isolated by gel filtration using sephadex(G-50 and G-100) that produced by ammonium sulphate precipitation. The apparent molecular weight of the isolated protein as a source of enzyme using gel filtration chromatography (G-100) was (75219 ± 900) Dalton for camel milk and (76493 ± 1000) Dalton for buffalo milk.

The results showed that the purified LP from camel and buffalo milk were at (70 µg/ml) of enzyme concentration using (0.08 mol/l) phosphate buffer at pH (7) act for (25) minutes at (45°C) and (400 mmol/l) of pyrogallol as a substrate. Using Line Weaver-Burk plot, the values of maximum velocity (V_{max}) and Michaelis constant (K_m) were found to be (3.49 µmol/ min) and (26.3 mmol/l) respectively for camel milk and (4.44 µmol/ min) (45.4 mmol/l) for buffalo milk.

Finally, the research was, also, involve the study of the effect of some chemicals and drugs on the enzyme activity. The results showed the sodium meta arsenite ($NaAsO_2$) uncompetitive inhibitor while phenylphrine is noncompetitive inhibitor for the enzyme at different concentrations of inhibitor, but disodium ethylene diamine tetra acetic acid (Na_2 -EDTA) and tetracycline were activators to LP.

Keywords: Separation, lactoperoxidase (LP), milk, camel, buffalo, inhibitors, activators.

(1881) Lactoperoxidase(LP) (EC 1.11.1.7)

(Ekstrand, 1994)

Reiter and)

(Harnulv,1984

Glycoprotein

Monoimmune

(Conner *et al.*, 2002)

LP

.Gram negative

Bactericidal

(H_2O_2)

.....

(Jooyandeh *et al.*,2011) Lactoperoxidase system(LPS)

(Adam *et al.*, 2008; Sharma *et al.*, 2009; Yener *et*

(100-75) LP *al.*,2009)

LP .(Juven and Pierson, 1996)

(`OSCN) (SCN`)

(Gülçin, 2009, 2010; Gülçin *et al.*, 2009)

(Seifu *et al.*, 2005)

%1

(Claesson, 1996) (FAO)

(WHO)

.(Saadde Schoos *et al.*, 1999)

(Shamsia, 2009)

Immunoglobulins

Antimicrobial factors

(LP)

.(De Wit and Van Hooydonk, 1996) 20

LP

LP

.()

:

:

.1

(/ /) Cooling centrifuge separation

(Ashiq, 1993)

40 3000 x g

()

Protein precipitation by salting out .2

%70

(Harvey and Ferrier, 2011)

° 4

24

Cooling centrifuge separation .3

6000 x g

30

LP

(Schacterle and Pollack, 1973)

.(Tayefi-Nasrabadi *et al.*, 2011)

° (20-)

Dialysis () .4

°4

(Boyer, 2000) /

0.1

6.8

3

24

G-50 .5

(2.6 × 90)

30

(Sephadex G-50) G-50

) Internal volume (V_i)

76

Blue

) Void volume (V_o)

((Trp.)

.(Murray *et al.*, 2009)

(dextran

Lyophilizer .6

LP

Sephadex G-50

° (20-)

.....

G-100

.7

(2.6 × 90)

100

(Sephadex G-100) G-100

76

)

(

.(Burtis *et al.*, 2012)

()

:

(

)

4

(Trp)

(

)

5

Blue dextran

Internal volume (V_i)

) LP

Void volume (V_o)

.(Papain

Pepsin

Egg albumin

Bovine serum albumin (BSA)

10 ()

0.1

5

.Elution solution

/ 160

5

/ 60

Fraction collector

(UV-VIS Spectrophotometer PD-303UV)

LP

280

Lyophilizer

.8

LP

Tayefi-Nasrabadi *et al.*,) :LP
 Pyrogallol LP (2011)
 Purpurogallin



0.1 6.5 pH -1
 10 Pyrogallol 200 -2
 61 -3
 150 3 -4
 50
 °24
 ° 24 430

LP

LP () (Mehrin *et al.*, 2011)
 (2 1)

(14000) (Murray *et al.*, 2009)
 G-100 G-50
 (194) (582)

.....

:

()
 G-100 Sephadex G-50
) (G-50)
 G -100 (2 1
 LP (4 3)
 .Sephadex G-100
 103.4 106.1
 .(3)
 (4 3)

Jooyandeh *et al.*, 2011; De)

.(Alba *et al.*, 2013

: 1

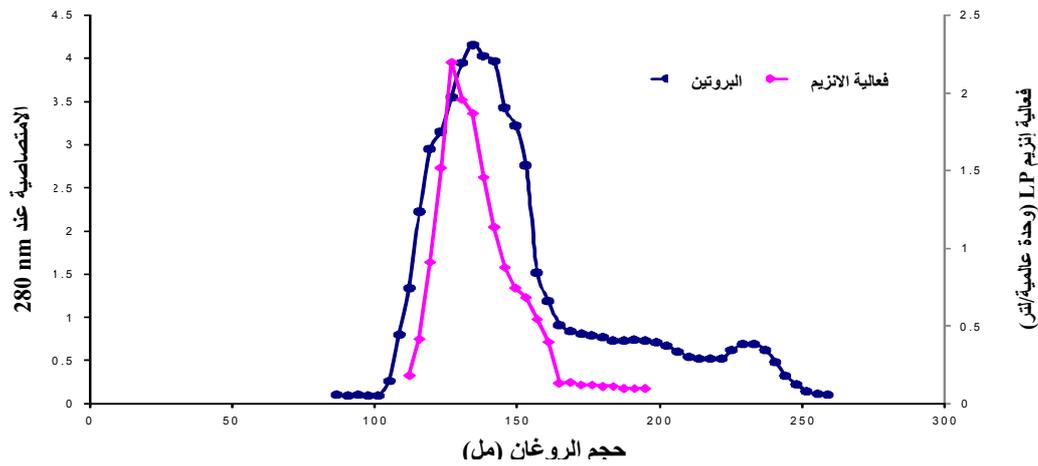
%		(/)	()	(/ *)	(/)	()	
100	1	0.016	349.895	0.5383	33.418	650	
98.3	1.7	0.0265	343.94	0.593	28.272	580	()
							%70
7.7	0.5	0.0071	27.11	0.0438	6.145	619	
45.7	7.6	0.122	160.0	2.963	24.315	54	
43.5	9.1	0.1456	152.11	2.012	13.818	75.6	
31.5	54	0.8653	110.25	1.4318	1.6545	77	G-50
10.5	194	3.1102	36.912	1.538	0.4945	24	G-100

: *

: 2

%		(/)	()	(/ *)	(/)	()	
100	1	0.0121	331.1	0.473	39.2	700	
95.5	1.3	0.0161	316.41	0.597	36.927	530	()
							%70
7.1	1.1	0.0129	23.49	0.0522	4.045	450	
57.5	3.0	0.0358	190.245	1.153	32.241	165	
56.4	4.0	0.0486	186.772	0.881	18.12	212	
35.5	115	1.3869	117.558	0.933	0.6727	126	G-50
34.6	582	7.041	114.75	2.125	0.3018	54	G-100

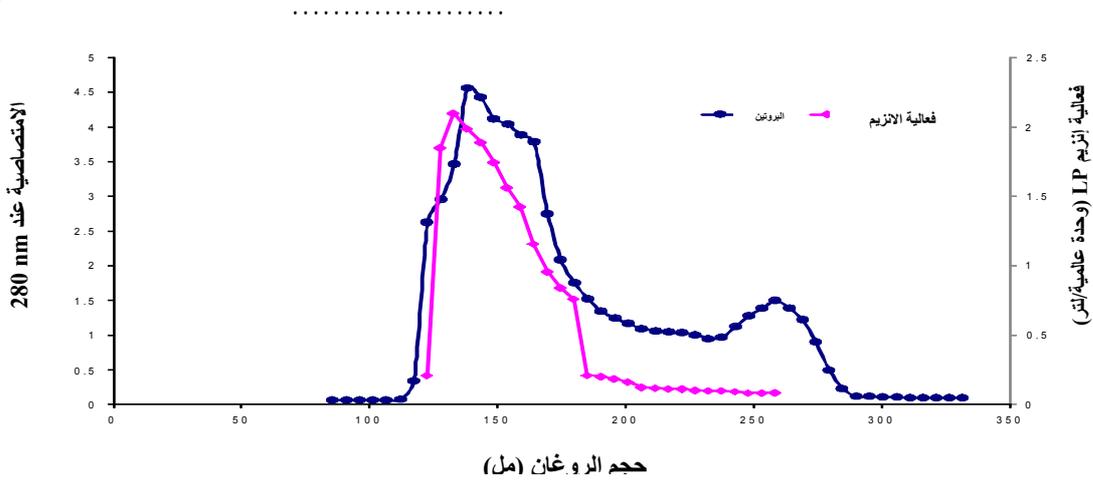
*



LP

:1

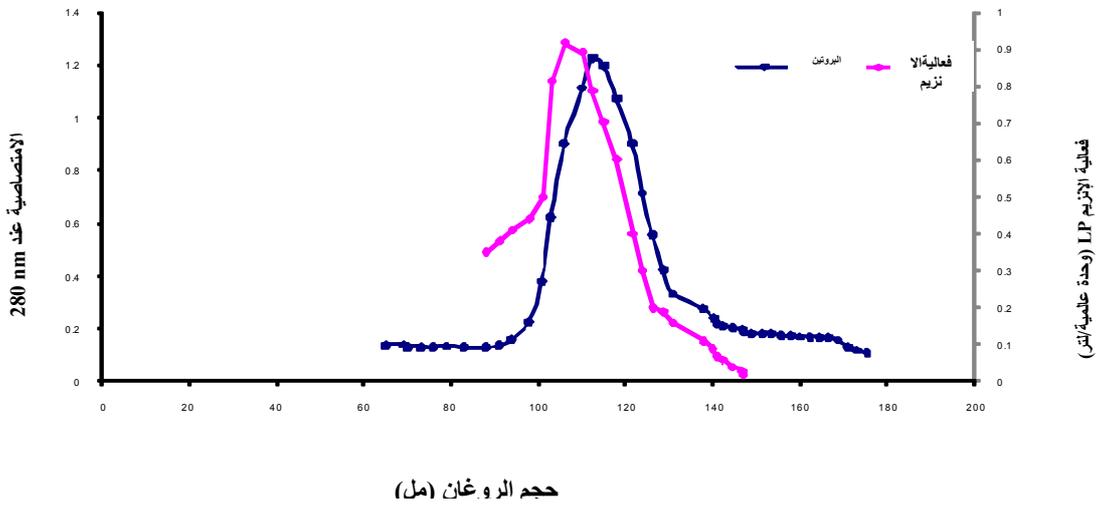
Sephadex G-50



LP

: 2

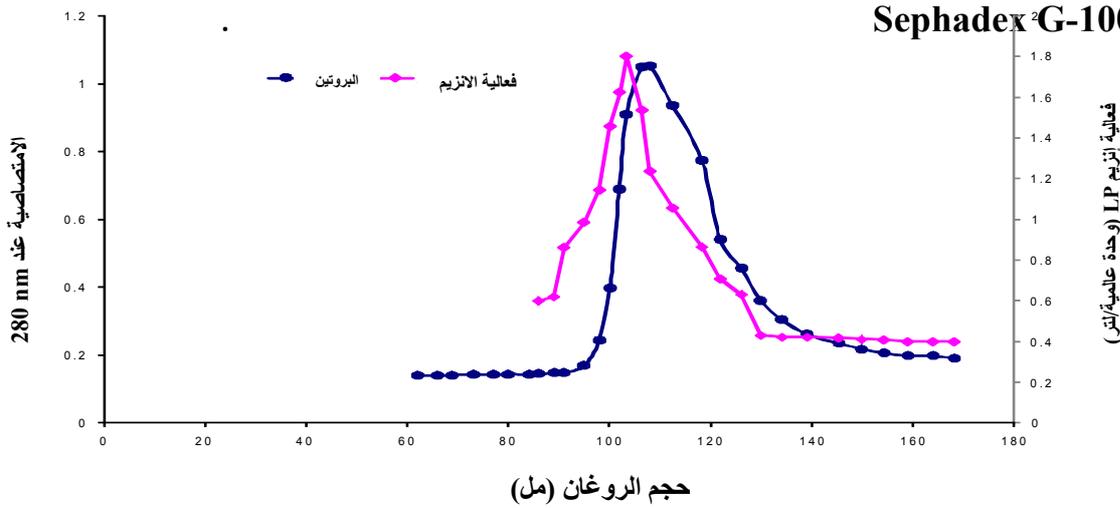
Sephadex G-50



LP

: 3

Sephadex²G-100



LP

: 4

Sephadex G-100

:

Sephadex G-100

(194)

(582)

(2000000 - 204)

(3) .(V_o)

(V_i)

(Sephadex G-100)

: 3

.Sephadex G-100

()	()	
91.2	2000000	Blue dextran
128.4	67000	Bovine serum albumin
175.9	45000	Egg albumin
229.2	36000	Pepsin
307.1	21000	Papain
412.1	204	Tryptophan
106.1*	75219	()
103.4**	76493	()

(3)

*

(4)

** .G-100

.G-100

(5)

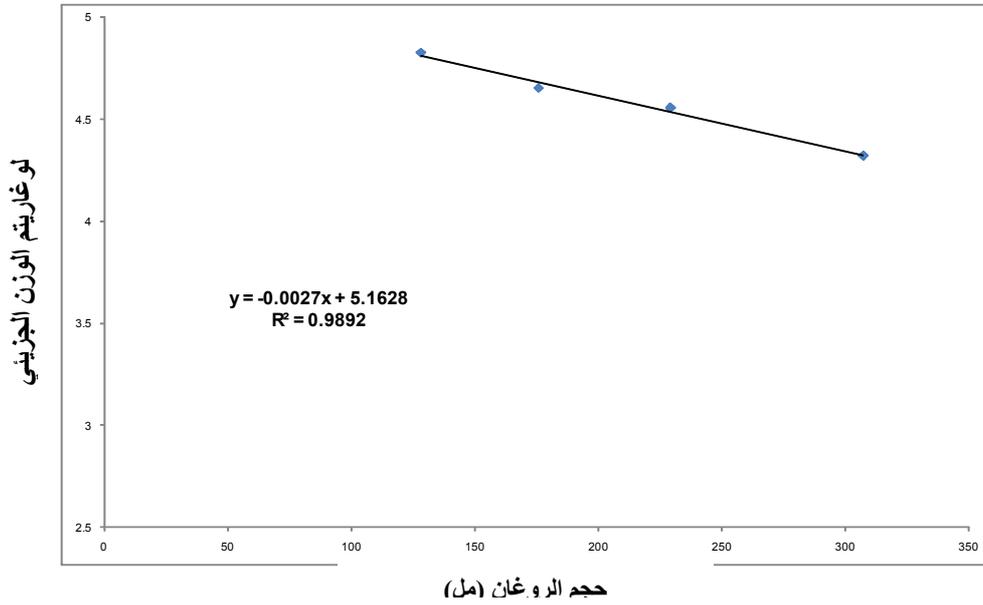
(900 ± 75219)

(1000 ±76493) (Mehrin *et al.*, 2011)

(78)

(Tanaka *et al.*, 2008)

.....



:5

.Sephadex G-100

LP

pH

.(Burtis et al., 2012)

.1

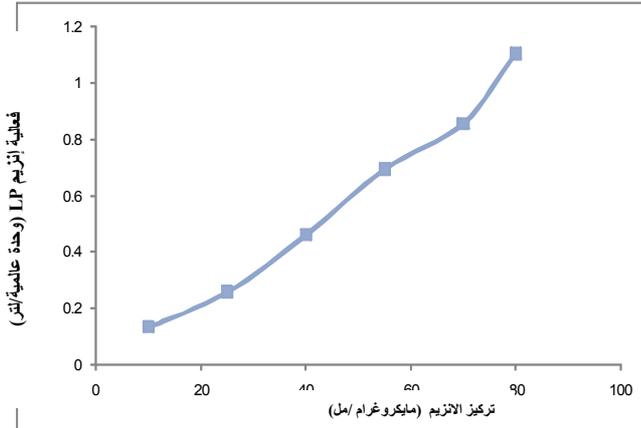
LP

:

(/ 80 -10)

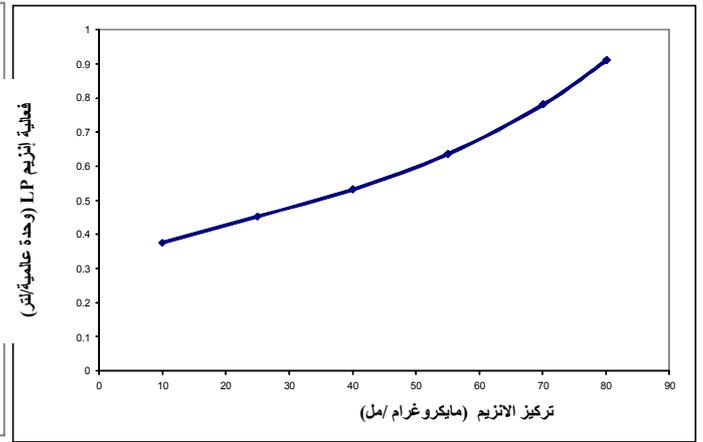
(7 6)

/ 70



LP

:7



LP

:6

.2

.1.2

(0.22-0.02)

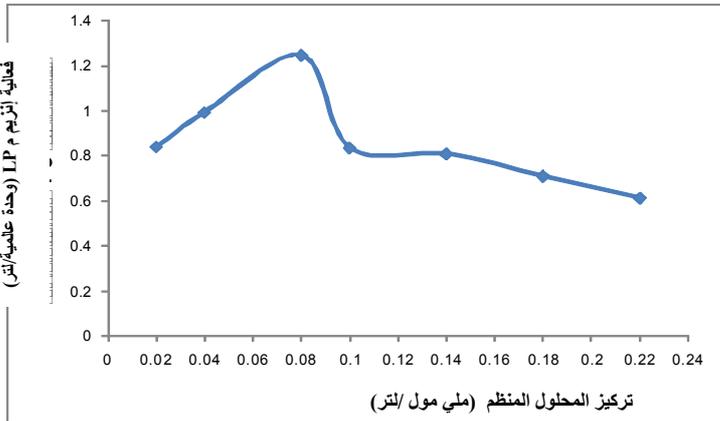
LP

6.5

/

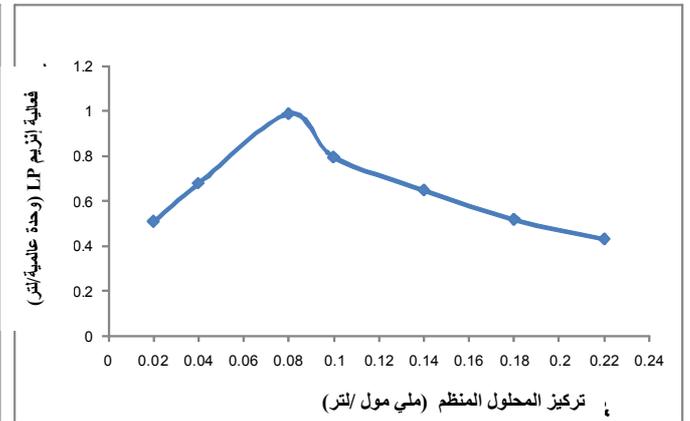
(9 8)

(/ 0.08)



LP

:9



LP

:8

.2.2

(pH)

.Optimum pH

0.08

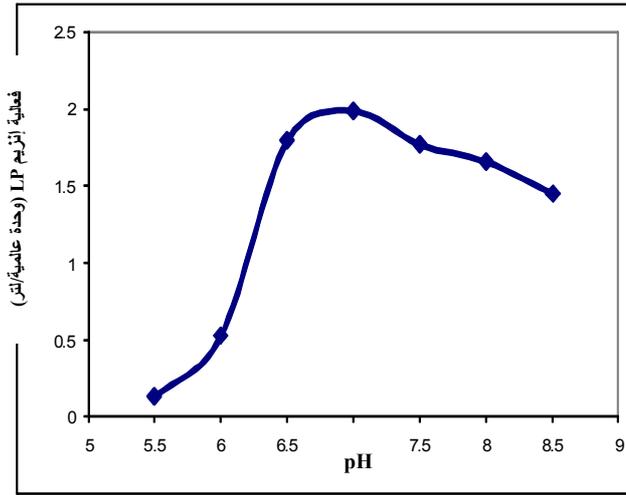
LP

7

(11 10)

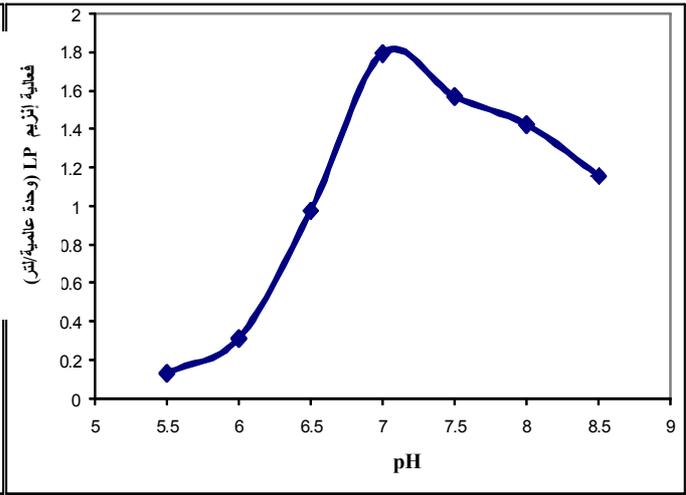
(Han et al., 2007)

.6.65



LP

:11



LP

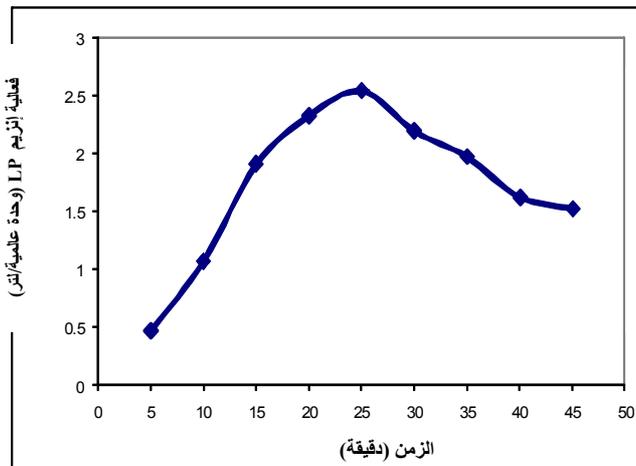
:10

:

.3

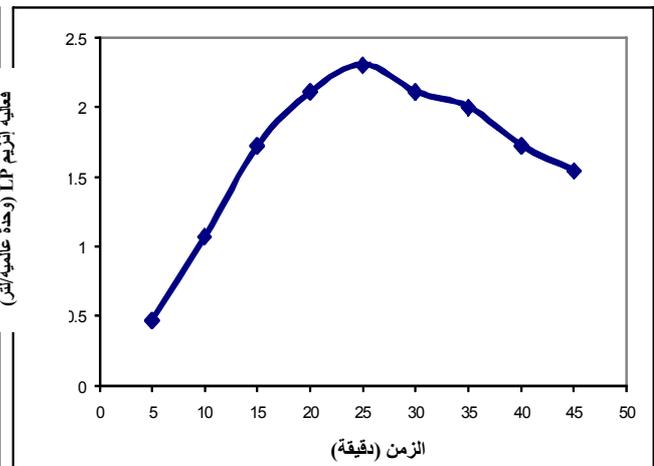
25

(13 12)



LP

:13



LP

:12

.4

:

)

(

(Harvey and Ferrier, 2011)

.(15 14)

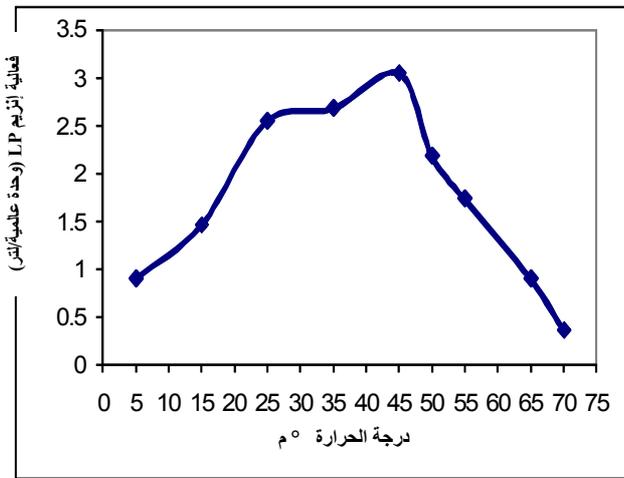
LP

°45

(Claeys *et al.*, 2002)

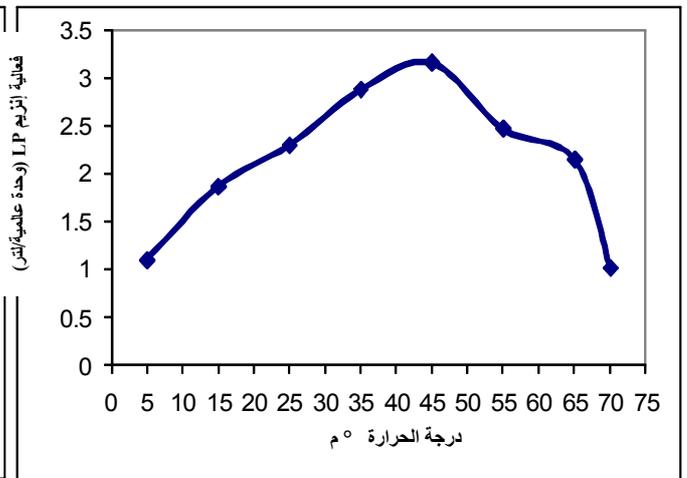
. ° 80

.(15 - 6)



LP

:15



LP

:14

.5

:

(Burtis *et al.*, 2012) (V_{max})

. / (600-50) ()

()

(18 16)

.....

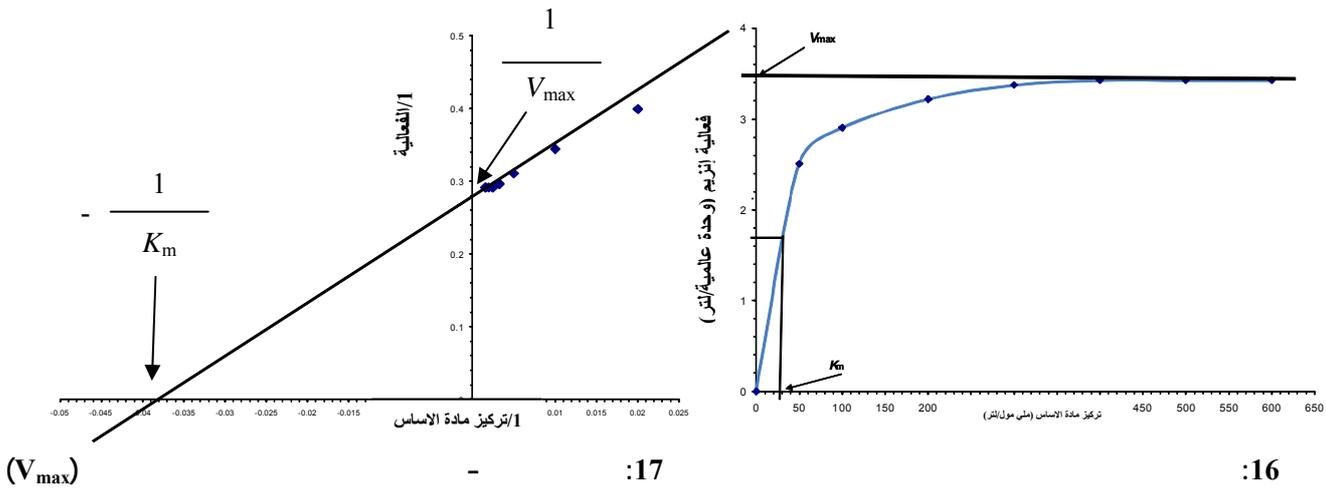
(V_{max})

(18 16) (Murray *et al.*, 2009)

/ 400

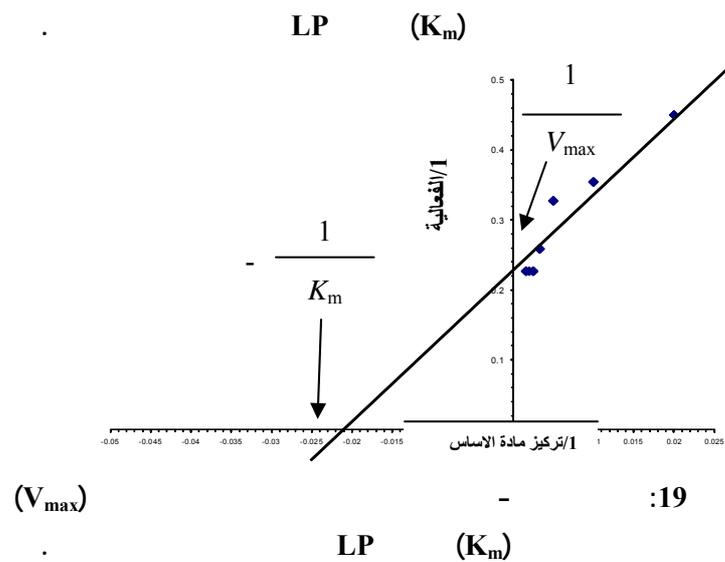
LP (K_m) (V_{max}) (Line Weaver-Burk plot)
 .(17) / 26.3 / 3.49
 / 45.4 / 4.44

.(19)



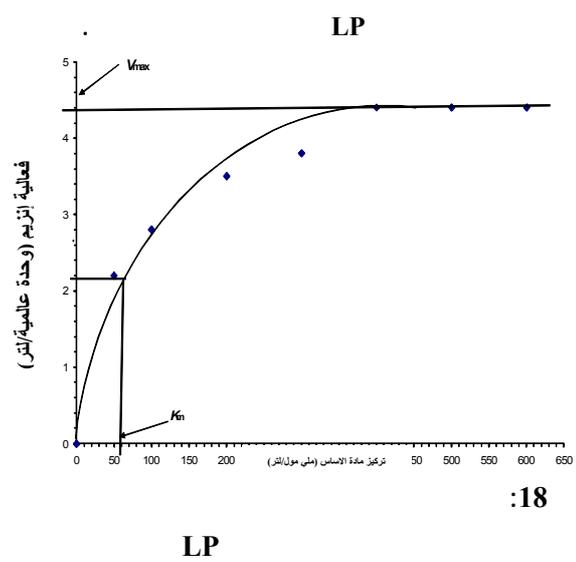
(V_{max}) :17

:16



(V_{max}) :19

:18



LP

LP (K_m)

:
LP
LP

582

(/ 500 400 300)
(4)
(1.7)

LP

NaAsO₂

Na₂-EDTA

LP

:4

(-) (+)	*	LP (/)	(/)	
-28.9		3.156	300	ZnSO ₄
-33.9		2.935	400	
-36.86		2.803	500	
-31.44		3.044	300	MgSO ₄
-39.1		2.704	400	
-48.51		2.286	500	
-32.86		2.981	300	NaF
-40.36		2.648	400	
-51.05		2.173	500	
-45.8		2.406	300	NaAsO ₂
-44.63		2.458	400	
-51.1		2.171	500	
+27.7		5.67	300	Na ₂ - EDTA
+34.3		5.963	400	
+41.37		6.277	500	

./ (4.44)

*

.....

:() .2.6

5 .LP
(/ 500 400 300)

(Neomycin Flagyl Ceramide)
(Falagas *et al.*, 2008; Horak *et al.*, 2009)

(Pacher *et al.*, 2006) Allopurinol

Paracetamol (Nehlig *et al.*, 1992)

Pseudoephedrine Phenylphrine
(Brunton, 2006; Horak *et al.*, 2009)

.(Yoshikawa, 2007) Theophylline anhydrous
(5)

.(3.7) (2.7)

()

) Prosthetic group

LP (Murray *et al.*, 2009) (

LP

.(Sheikh *et al.*, 2009; Singh *et al.*, 2009)

. LP .7

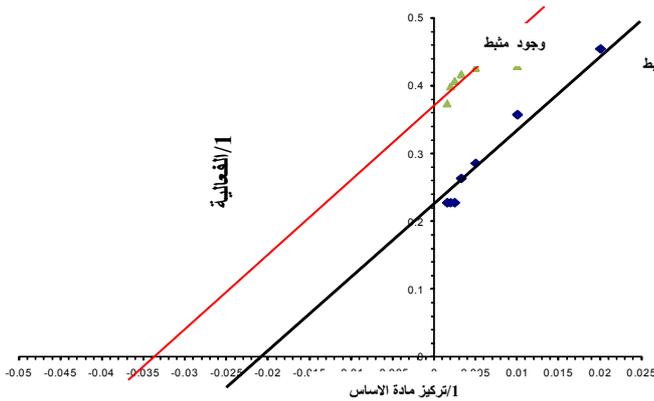
: .1.7

– .(20)

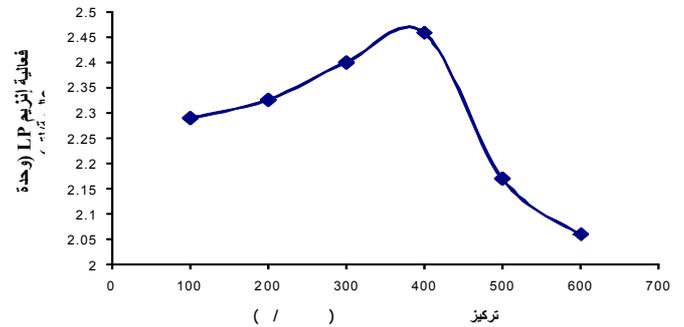
Uncompetitive inhibitor

V_{max}

.(21) (29.41) (2.7) (Burtis *et al.*, 2012) K_m



الشكل 21: رسم لاينوفير- برك عند استخدام المثبط ميتا زرنبيخت الصوديوم.



الشكل 20 : تأثير تركيز مادة انزيم LP.

الشكل 20 : تأثير تركيز مادة انزيم LP.

LP

:5

*النسبة المئوية المنوية للتثبيط (-) أو التنشيط (+)	فعالية الإنزيم LP (وحدة عالمية/لتر)	تركيز المواد (ملي مول/لتر)	الأدوية
- 31.44	3.044	300	كالامين Calamine
- 35.2	2.877	400	
- 46.46	2.377	500	
-32.95	2.977	300	الويورينول Allopuranol
-36.86	2.803	400	
-40.65	2.635	500	باراسيتامول Paracetamol
- 33.44	2.955	300	
- 41.95	2.577	400	
- 48.1	2.304	500	ثايوفيلين اللاماني Theophylline anhydrous
- 27.86	3.203	300	
- 31.5	3.041	400	
- 36.59	2.815	500	الكافيين اللاماني Caffein anhydrous
-39.12	2.703	300	
- 40.51	2.641	400	
- 46.46	2.377	500	فينايل فرين Phenylphrine
- 41.68	2.589	300	
-42.27	2.563	400	
-45.81	2.406	500	فلاجيل Flagyl
-26.37	3.269	300	
-31.44	3.044	400	
-39.09	2.704	500	سيراميد Ceramide
-52.52	2.108	300	
-59.36	1.804	400	
-66.5	1.487	500	بسيديوالايفيدرين Pseudephidrine
-33.85	2.937	300	
-38.76	2.719	400	
-44.21	2.477	500	نيومايسين Neomycin
-31.44	3.044	300	
-37.56	2.772	400	
-41.75	2.586	500	تتراسايكلين Tetracycline
+ 2.9	4.569	300	
+ 8.17	4.803	400	
+ 23.08	5.465	500	

* قيمة الفعالية القياسية للإنزيم هي (4.44) وحدة عالمية / لتر.

2.7. مادة فينايل فرين :

(22)

Noncompetitive

inhibitor

K_m

(2.94)

V_{max}

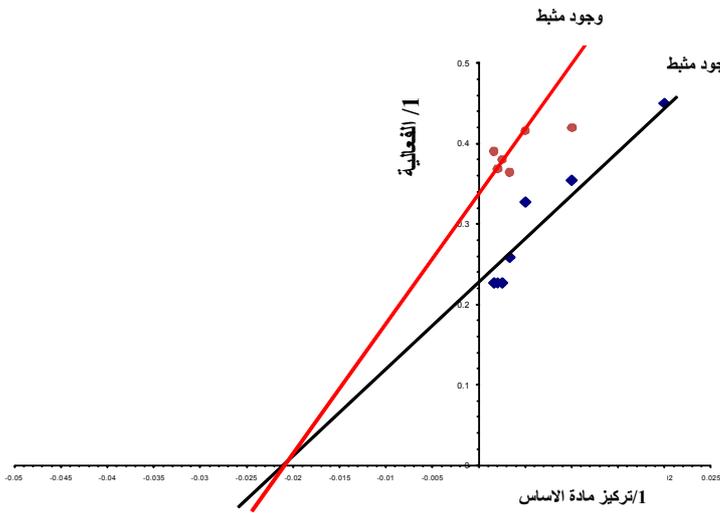
.23

(Burtis *et al.*, 2012)

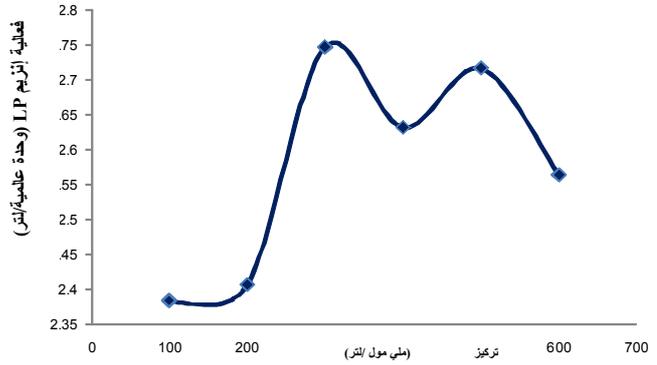
LP

Sulphanilamide

(Atasever *et al.*, 2013)



: 23



: 22

.LP

:

(Na_2-EDTA)

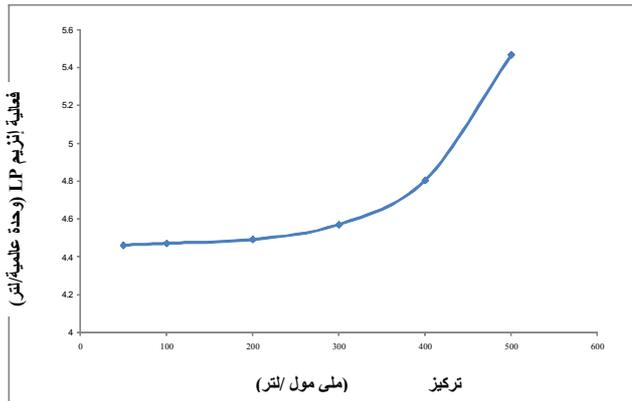
3.7. تأثير

LP

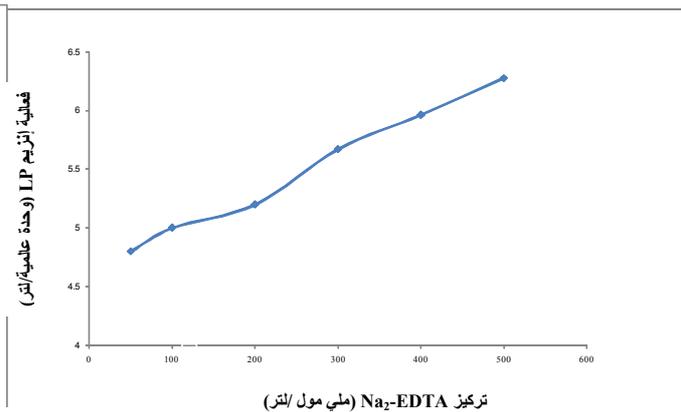
Na_2-EDTA

(25 24)

LP



.LP :25



.LP Na₂-EDTA : 24

() Activators

(Dimitrov *et al.*, 2007)

(Burtis *et al.*, 2012)

Monoaminomonocarbonic acid

LP

Diaminodicarbonic acid

%2.77 LP

. %2.17

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